Seroepidemiologic Survey of Crimean-Congo Hemorrhagic Fever Virus in Logging Communities, Myanmar

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Crimean-Congo hemorrhagic fever virus (CCHFV) is endemic in Asia, infecting many animal hosts, but CCHFV has not been reported in Myanmar. We conducted a sero-epidemiologic survey of logging communities in Myanmar and found CCHFV exposure was common (9.8%) and exposure to wild animal blood and body fluids was associated with seropositivity.

Crimean-Congo hemorrhagic fever (CCHF), caused by Crimean-Congo hemorrhagic fever virus (CCHFV) (1), is a widely distributed arboviral disease. Human CCHF cases have been reported in >30 countries in Africa, the Middle East, Asia, and southeastern Europe (2). However, clinical cases or seroprevalence studies for CCHFV have not been reported in Myanmar, likely because active surveillance in humans or animals has not been established (3).

Hyalomma ticks, 1 of several CCHFV tick family hosts, are considered the primary vector transmitting CCHFV to humans (4). Hyalomma tick distribution extends into Myanmar (5), and CCHF has been reported in the neighboring countries of China and India (6,7). Expansion of CCHF from countries with known

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virus circulation to neighboring countries could occur through introduction of infected ticks, human CCHF cases, or movement of animals (8). Climate change also is expected to influence the distribution of *Hyalomma* ticks and CCHFV infections (9), increasing the likelihood of disease expansion.

Human CCHFV infections can occur through contact with an infected tick or with blood or tissues from infected humans or animals. People living or working closely with livestock or who have heavy exposure to ticks are at increased risk for CCHFV infection (10,11). Limited investigations have been performed to identify human exposure to CCHFV caused by wild animal contact, despite serologic evidence for exposure to CCHFV in numerous vertebrate species, including birds (Galliformes and Passeriformes), wild hoof stock (Artiodactyla, Cetartiodactyla, and Perissodactyla), carnivores (Carnivora), bats (Chiroptera), hedgehogs (Erinaceomorpha), rabbits and hares (Lagomorpha), elephants (Proboscidea), rodents (Rodentia), and turtles (Testudinata) (12).

CCHF has been designated by the World Health Organization as 1 of 10 high-priority emerging infectious diseases (https://www.who.int/emergencies/diseases/2018prioritization-report.pdf). The designation was based on CCHF's epidemic and emergence potential, a high case-fatality rate of up to 80% depending on healthcare infrastructure and CCHFV genotype, and a lack of approved medical countermeasures for CCHF (14). Most initial reports of CCHF cases in individual countries have been preceded by epidemiologic surveys that provided evidence of local CCHFV circulation. Our goal was to conduct

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targeted CCHFV surveillance of Myanmar logging communities, which contain an occupational group with expected high exposure to ticks, domestic livestock, and wild animals.

The Study

Myanmar uses a traditional method of elephant logging for timber harvest. Consequently, Myanmar has a large network of communities in which loggers live together in temporary villages with their families and occasionally migrant laborers. We collected data from 102 healthy persons from 5 elephant logging communities in and near the Yenwe Forest Reserve, a protected area in central Myanmar, during June 2016-August 2018. Most (57/102) participants, including persons from forest management, logging crews, and elephant caretakers, worked in the protected area and were exposed to forested areas and vectors associated with CCHFV (Table 1). Participants were 17-67 years of age and the median age was 32.5 years. We collected venous blood samples and quantitative medical and behavioral questionnaires from each participant (Appendix, https://wwwnc.cdc.gov/EID/ article/27/6/20-3223-App1.pdf).

We used a bead-based MagPix (Luminex Corporation, https://www.luminexcorp.com) assay platform, developed at the US Army Medical Research Institute of Infectious Diseases, to detect specific IgG reactivity against the nucleoprotein of CCHFV. We used molecular detection of conserved

regions of the small, medium, and large segments of bunyavirus to detect CCHFV viremia with conventional PCR (Appendix).

We identified previous CCHFV exposure among study participants, but we did not detect any active infections. Study participants did not exhibit any signs of hemorrhagic fever, and none reported having previously suffered symptoms of hemorrhagic-like illnesses. All participants tested negative for bunyaviruses by consensus PCR. Among study participants, 9.8% (10/102) were seropositive for CCHFV by Mag-Pix IgG assay. Samples categorized as positive ranged from 1,124-8,911 mean fold increase (MFI) and a signal-to-noise ratio (S/N) of 33.8-207.8. Negative samples had an MFI of 44-854 and 1-19.9 S/N. Persons 31-40 years of age were significantly more likely to be seropositive for CCHFV (p = 0.05) compared with other age groups. We noted no statistically significant associations between specific occupations and CCH-FV exposure (Table 1).

Persons who reported handling live or recently slaughtered primates (age-adjusted odds ratio $[OR_{age\ adjusted}] = 5.53$; p = 0.020) or wild carnivores $(OR_{age\ adjusted}] = 1.3$; p = 0.004) in their lifetimes were more likely to have been exposed to CCHFV (Table 2). Handling primates was significantly correlated with handling carnivores (Pearson's correlation = 0.6; p<0.001). Therefore, we used independent multivariable logistic regression models to adjust for age while assessing the association of CCHFV seropositivity for these 2 factors. More male than female persons reported

Table 1. Crimean-Congo hemorrhagic fever virus immunoglobulin G seroprevalence by demographic characteristic and occupation among forest logging camp communities, Myanmar

Characteristic	No. positive	No. negative	Period prevalence (95% CI)
Sex			
M	6	56	0.11 (0.05–0.2)
F	4	36	0.11 (0.04–0.23)
Age group, y			
11–20	0	11	0 (0–0.26)
21–30	3	32	0.09 (0.03-0.22)
31–40	5	19	0.21 (0.09–0.40)
41–50	2	13	0.13 (0.04–0.38)
51–60	0	15	0 (0–0.20)
61–70	0	2	0 (0–0.66)
Primary occupation*			· ·
Extractive industries	0	6	0 (0-0.39)
Crop production	0	2	0 (0–0.66)
Livestock farmer	0	1	0 (0–0.79)
Protected area worker, forest ranger	6	51	0.11 (0.05–0.21)
Housewife	1	2	0.33 (0.06–0.79)
Teacher	0	2	0 (0-0.66)
Migrant laborer	0	5	0 (0–0.43)
Hunter	1	7	0.11 (0.02–0.43)
Dependent	3	27	0.09 (0.03–0.24)
Total	10	92	0.11 (0.05–0.17)

^{*}Persons were asked to report their primary occupations but some engaged in additional activities, outside of their primary occupation. For example, persons who did not identify as being a hunter as their primary occupation may have reported hunting.

Table 2. Distribution of seropositivity to Crimean-Congo hemorrhagic fever virus among persons exposed to wild and domesticated

animals in	forest loggin	d camp	communities.	Mvanmar*

	Exposed no. persons	Unexposed no. persons	Bivariate model		Multivaria	
Risk factor	seropositive/no. tested (%)	seropositive/no. tested (%)	OR	p value	OR	p value
Hunted wildlife						
Ungulate	2/27 (7.4)	8/75 (10.7)	0.67	1.0	NC	NC
Bat	0/1 (0.0)	10/101 (9.9)	2.9†	1.0	NC	NC
Rodent	0/1 (0.0)	10/101 (9.9)	2.9†	1.0	NC	NC
Primate	2/16 (12.5)	8/86 (9.3)	1.39	0.66	NC	NC
Pangolin	2/9 (22.2)	8/93 (8.6)	2.99	0.21	NC	NC
Carnivore	1/9 (11.1)	9/93 (9.7)	1.16	1.0	NC	NC
Any wild animal	4/51 (7.8)	6/51 (11.8)	0.64	0.74	NC	NC
Handled wildlife found dead						
Ungulate	3/32 (9.4)	7/70 (10.0)	0.93	1.0	NC	NC
Bat	1/3 (33.3)	9/99 (9.1)	4.86	0.27	NC	NC
Rodent	1/4 (25.0)	9/98 (9.2)	3.24	0.34	NC	NC
Primate	4/19 (21.1)	6/83 (7.2)	3.37	0.09	NC	NC
Pangolin	1/6 (16.7)	9/96 (9.4)	1.92	0.47	NC	NC
Carnivore	1/10 (10.0)	9/92 (9.8)	1.02	1.0	NC	NC
Any wild animal	8/76 (10.5)	2/26 (7.7)	1.41	1.0	NC	NC
Handled recently slaughtered	or live wildlife					
Ungulate	4/26 (15.4)	6/76 (7.9)	2.1	0.27	NC	NC
Bat	1/3 (33.3)	9/99 (9.1	4.86	0.27	NC	NC
Rodent	1/5 (20.0)	9/97 (9.3)	2.42	0.41	NC	NC
Primate	5/23 (21.7)	5/79 (6.3)	4.04	0.04	5.53‡	0.020
Pangolin	2/10 (20.0)	8/92 (8.7)	2.59	0.25	NC	NC
Carnivore	4/12 (33.3)	6/90 (6.7)	6.78	0.02	1.3‡	0.004
Any wild animal	10/88 (11.4)	0/14 (0.0)	3.88†	0.35	NC	NC
Handled live domestic animals	3					
Goats	0/6 (0.0)	10/96 (10.4)	0.63†	1.0	NC	NC
Pigs	3/23 (13.0)	7/79 (8.9)	1.54	0.69	NC	NC
Poultry	6/57 (10.5)	4/45 (8.9)	1.20	1.0	NC	NC
Cattle	1/8 (12.5) [´]	9/94 (9.6)	1.34	0.58	NC	NC
Elephant	5/43 (11.6)	5/59 (8.5)	1.42	0.74	NC	NC
Any domestic animal	7/60 (11.7)	3/42 (7.1)	1.71	0.52	NC	NC
Slaughtered domestic animals	, ,	,				
Goats	0/0 (0.0)	10/102 (9.8)	NC	NC	NC	NC
Pigs	1/3 (33.3)	9/99 (9.1)	4.86	0.27	NC	NC
Poultry	3/18 (16.7)	7/84 (8.3)	2.18	0.38	NC	NC
Cattle	0/1 (0.0)	10/101 (9.9)	2.9†	1.0	NC	NC
Any domestic animal	9/71 (12.7)	1/31 (3.2)	4.31	0.28	NC	NC

^{*}NC, not calculated; OR, odds ratio.

handling primates (20 male vs. 3 female persons) and carnivores (9 male vs. 3 female persons) and their ages ranged from 19–60 years. Handling primates or carnivores was not statistically significantly associated with any occupational or other behavioral factors.

Among persons who reported handling wildlife, the highest risk species for CCHFV exposure were primates and carnivores. Although sample size for handling some live or recently slaughtered wild animal taxa were low (for instance, <5 persons each reported handling rodents or bats), we found no statistically significant association between combined wildlife taxa evaluated and CCHFV exposure (p = 1.0; Table 2).

A bite from an infected tick was not the likely route of exposure to CCHFV in this community. We evaluated occupations associated with increased forest contact, and thus tick habitat, such as resource extraction, protected area worker (forest ranger), or hunter, as a combined variable, but we found no statistically significant association between occupation and CCHFV exposure.

Contact with domestic animals also was not the likely route of CCHFV exposure in this community. Study participants were not frequently exposed to ruminants, the domestic animal group most reported as associated with CCHFV exposure in endemic countries. Participants were more likely to report contact with pigs or poultry, but these animals have not been identified as amplifying hosts for CCHFV. Contact with live or dead domestic animals of any kind was not associated with CCHFV exposure (Table 2).

Nonhuman primates have not been implicated as natural reservoir hosts or sources of human CCHFV infection. However, rhesus macaques (*Macaca mulatta*) and long-tailed macaques (*M. fascicularis*), which

[†]Sample odds ratio calculated using unconditional maximum likelihood estimate method

[‡]Evaluated in separate multivariable models, adjusting for age.

range throughout Myanmar, have been infected with CCHFV in laboratory settings. Rhesus macaques develop viremia without clinical signs, but long-tailed macaques develop signs of clinical illness and viremia similar to disease progression in humans (15). Contact with blood or other bodily fluids, including saliva, urine, or feces, during a period of viremia in macaques could lead to human infection. Similarly, wild carnivores have not been implicated as natural reservoir hosts for CCHFV, but red foxes (Vulpes vulpes), which are thought to range in Myanmar, and Pallas's cats (Otocolobus manul), which range in central Asia, have demonstrated CCHFV seropositivity and could serve as sources of human infection, particularly through bushmeat hunting, which exposes persons to animal blood and body fluids.

Conclusions

Our findings indicate that CCHFV is circulating in Myanmar with human infections that are either mildly symptomatic or occurring in populations that fall outside of existing surveillance systems. Although exposure to domestic animal amplifying hosts is the most commonly reported exposure type for human CCHFV infections in endemic countries, our findings show that persons with close contact with wild animal reservoir hosts, especially blood and body fluids of nonhuman primates and carnivores, also are at risk for CCHFV infection. Surveillance of at-risk populations in Myanmar should be expanded to better prepare for potential future outbreaks of CCHF.

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References

- Hoogstraal H. The epidemiology of tick-borne Crimean-Congo hemorrhagic fever in Asia, Europe, and Africa. J Med Entomol. 1979;15:307–417. https://doi.org/ 10.1093/jmedent/15.4.307
- Ergönül O. Crimean-Congo haemorrhagic fever. Lancet Infect Dis. 2006;6:203–14. https://doi.org/10.1016/ S1473-3099(06)70435-2
- Al-Abri SS, Abaidani IA, Fazlalipour M, Mostafavi E, Leblebicioglu H, Pshenichnaya N, et al. Current status of Crimean-Congo haemorrhagic fever in the World Health Organization Eastern Mediterranean Region: issues, challenges, and future directions. Int J Infect Dis. 2017;58:82– 9. https://doi.org/10.1016/j.ijid.2017.02.018
- Maltezou HC, Papa A. Crimean-Congo hemorrhagic fever: risk for emergence of new endemic foci in Europe? Travel Med Infect Dis. 2010;8:139–43. https://doi.org/10.1016/ j.tmaid.2010.04.008
- Messina JP, Pigott DM, Golding N, Duda KA, Brownstein JS, Weiss DJ, et al. The global distribution of Crimean-Congo hemorrhagic fever. Trans R Soc Trop Med Hyg. 2015;109:503–13. https://doi.org/10.1093/trstmh/trv050
- Bente DA, Forrester NL, Watts DM, McAuley AJ, Whitehouse CA, Bray M. Crimean-Congo hemorrhagic fever: history, epidemiology, pathogenesis, clinical syndrome and genetic diversity. Antiviral Res. 2013;100:159–89. https://doi.org/10.1016/j.antiviral.2013.07.006
- Patel AK, Patel KK, Mehta M, Parikh TM, Toshniwal H, Patel K. First Crimean-Congo hemorrhagic fever outbreak in India. J Assoc Physicians India. 2011;59:585–9.
- Spengler JR, Bergeron É, Spiropoulou CF. Crimean-Congo hemorrhagic fever and expansion from endemic regions. Curr Opin Virol. 2019;34:70–8. https://doi.org/10.1016/ j.coviro.2018.12.002
- Estrada-Peña A, Sánchez N, Estrada-Sánchez A. An assessment of the distribution and spread of the tick *Hyalomma marginatum* in the western Palearctic under different climate scenarios. Vector Borne Zoonotic Dis. 2012;12:758–68. https://doi.org/10.1089/ vbz.2011.0771
- Vorou R, Pierroutsakos IN, Maltezou HC. Crimean-Congo hemorrhagic fever. Curr Opin Infect Dis. 2007; 20:495–500. https://doi.org/10.1097/QCO.0b013e3282a 56a0a
- Deyde VM, Khristova ML, Rollin PE, Ksiazek TG, Nichol ST. Crimean-Congo hemorrhagic fever virus genomics and global diversity. J Virol. 2006;80:8834–42. https://doi.org/10.1128/JVI.00752-06
- 12. Spengler JR, Bergeron É, Rollin PE. Seroepidemiological studies of Crimean-Congo hemorrhagic fever virus in domestic and wild animals. PLoS Negl Trop Dis.

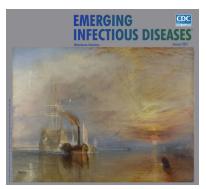
- $2016; 10: e0004210.\ https://doi.org/10.1371/journal.\ pntd.0004210$
- Schwarz TF, Nsanze H, Ameen AM. Clinical features of Crimean-Congo haemorrhagic fever in the United Arab Emirates. Infection. 1997;25:364–7. https://doi.org/10.1007/ BF01740819
- Leblebicioglu H, Ozaras R, Irmak H, Sencan I. Crimean-Congo hemorrhagic fever in Turkey: Current status and future challenges. Antiviral Res. 2016;126:21–34. https://doi.org/10.1016/j.antiviral.2015.12.003
- Smith DR, Shoemaker CJ, Zeng X, Garrison AR, Golden JW, Schellhase CW, et al. Persistent Crimean-Congo hemorrhagic fever virus infection in the testes and within granulomas of non-human primates with latent tuberculosis. PLoS Pathog. 2019; 15:e1008050. https://doi.org/10.1371/journal. ppat.1008050

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Seroepidemiologic Survey of Crimean-Congo Hemorrhagic Fever Virus in Logging Communities, Myanmar

Appendix

Project Approvals

Study protocols were reviewed independently and ethical approval was provided by the Institutional Review Board (approval no. 889159–2) and Institutional Animal Care and Use Committee (approval no. 19520) at the University of California, Davis, the Ethics Review Committee of the Department of Medical Research (approval no. 012816), the Forest Department of the Ministry of Natural Resources and Environmental Conservation, the Livestock Breeding and Veterinary Department and the Myanmar Timber Enterprise.

Bead-Based Serologic Assay

Specific immunoglobulin G (IgG) reactivity against Crimean-Congo hemorrhagic fever virus (CCHFV) was detected by using a bead-based assay, MagPix (Luminex Corporation, https://www.luminexcorp.com), developed at the U.S. Army Medical Research Institute of Infectious Diseases. MagPix has demonstrated an enhanced sensitivity profile relative to conventional ELISA (1,2) and detailed methods have been described previously by Smith et al. (3). In brief, recombinant CCHFV nucleoprotein, produced in a baculovirus expression system and based on the IbAr10200 isolate (GenBank accession no. KY484036) as a reference strain, were conjugated to magnetic microspheres by using the xMAP Antibody Coupling Reagent Kit (Luminex Corporation) according to the manufacturer's instructions. Antigen coupled beads were combined with 1:100 diluted serum and analyzed on the MagPix instrument. Data were evaluated as signal to noise (S/N), with noise being the average median fluorescence intensity of each bead set in response to naive serum samples. We considered any sample with S/N >20 to be seropositive.

Assay Validation

In a comparative study evaluating the immune response to both CCHFV strain Kosova Hoti (GenBank accession nos. DQ133507, EU037902, EU044832) and strain Afg09–2990 (GenBank accession nos. HM452307, HM452306, HM452305) in experimentally infected Cynomolgus macaques (*Macaca fascicularis*), host antibody response was measured from 1–28 days post CCHFV inoculation by viruses derived from both IgG MagPix assay and neutralization assay. Virus-neutralization response was evaluated by using a virus-like particle (VLP) system with glycoproteins based on CCHFV strain IbAr 10200. We observed the emergence of neutralizing antibodies in serum samples by day 9 post infection for both groups, with broadly similar kinetics and endpoint titers between the Hoti and Afg09 infected groups. Mean fold increase (MFI) values averaging 5,737 corresponded with an 80% plaque reduction neutralization titer (PRNT₈₀) of 1:100 and MFI values averaging 10,243 corresponded with a PRNT₈₀ of 1:400. MagPix and PRNT₈₀ values both peaked at day 21 post inoculation.

Determination of Cutoff Value

To determine cutoff values, MFI and S/N were evaluated for a large multiregional serum set, 1,614 samples from Africa and 634 from Asia. Cutoff's were conservatively set at S/N of 20, far exceeding standard serologic assay cutoff algorithms that would have used 3 standard deviations above the mean of the negative controls.

PCR Assay for Bunyaviral Small, Medium, and Large Segments

Samples were processed for viral detection by using consensus PCR, which enables the universal amplification of sequences from viruses within a given family or genus, and the subsequent discernment of viral strains within. Total nucleic acid was extracted from whole blood by using Direct-zol RNA Miniprep Kits (Zymo Research, https://www.zymoresearch.com) according to the manufacturer's instructions. Total RNA was reverse transcribed into complementary DNA (cDNA) by using SuperScript III (Invitrogen, https://www.thermofisher.com) according to the manufacturer's instructions, and 3 assays were used for detection of bunyaviral small, medium, and large segments as described previously by Briese, et al. (4).

Statistical Analyses

To evaluate associations between human demographic and animal contact behaviors, all demographic factors, including age, sex, and livelihood, were first evaluated for associations with animal contact behaviors to assess potential confounding. Fisher exact tests were used to determine associations between CCHFV exposure and demographic as well as high-risk human–animal contact behaviors. Odds ratios were calculated by using a conditional maximum likelihood estimate method. For variables, in which 0-count cells were present, we calculated odds ratios by using an unconditional maximum likelihood estimate, Haldane-Anscombe correction. We considered p<0.05 statistically significant. Then we used multivariable logistic regression to assess the association between high-risk wild animal contact behaviors and other risk factors that were significant on bivariate analysis. Variables were included when they significantly improved model fit, based on the likelihood ratio test (p<0.1), while minimizing the Akaike information criterion. Overall model fit was assessed by using the Hosmer-Lemeshow goodness-of-fit test. All statistical analyses were performed using R version 3.6.1 (R Foundation for Statistical Computing, https://www.r-project.org).

References

- Satterly NG, Voorhees MA, Ames AD, Schoepp RJ. Comparison of MagPix assays and enzyme-linked immunosorbent assay for detection of hemorrhagic fever viruses. J Clin Microbiol. 2016;55:68– 78. PubMed https://doi.org/10.1128/JCM.01693-16
- Ricks KM, Shoemaker CJ, Dupuy LC, Flusin O, Voorhees MA, Fulmer AN, et al. Development of a bead-based immunoassay using virus-like particles for detection of alphaviral humoral response. J Virol Methods. 2019;270:12–7. <u>PubMed https://doi.org/10.1016/j.jviromet.2019.04.013</u>
- Smith DR, Shoemaker CJ, Zeng X, Garrison AR, Golden JW, Schellhase CW, et al. Persistent Crimean-Congo hemorrhagic fever virus infection in the testes and within granulomas of non-human primates with latent tuberculosis. PLoS Pathog. 2019;15:e1008050. <u>PubMed https://doi.org/10.1371/journal.ppat.1008050</u>
- 4. Briese T, Kapoor V, Lipkin WI. Natural M-segment reassortment in Potosi and Main Drain viruses: implications for the evolution of orthobunyaviruses. Arch Virol. 2007;152:2237–47. PubMed https://doi.org/10.1007/s00705-007-1069-z

Human-Animal Contact Behavior Questionnaire

Partici	ipant ID:	
	e of Interview	
2 \Mb	ere are you conducting this interview?	
	e/City	
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Provin	nce/State	
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	riewer: Please collect GPS coordinates if administering using paper and	pen.
Intervi	iew/Questionnaire Begins	
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ii uie e	exact age is unknown, enter the respondent's estimated age.	
4. Wh	ere do you live?	
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Distric	xt	
Provin	nce/State	
	riewer: Probe for landmarks or nearest known site if area unknown. GPS etion of interview.	S coordinates to be identified and entered after
ООППРІ		<1 mo
	Harridana harra vari livad thara?	1 mo-1 y
5.	How long have you lived there?	>1–5 y
	Select one option.	>5–10 y
		>10 y
	w many in the dwelling are children less than 5 y old?	
_	Is the dwelling a permanent structure (that cannot be moved)?	yes
9.	,	no
		piped in water/water taps
		covered well
10.	Do you get water from: Select all that apply.	uncovered well/pond/river
	Select all that apply.	water truck/rainwater harvest
		other
4.4	De construe de construe de la live de construe O	yes
11.	Do you treat your drinking water?	no
		boil
		filter
12.	If yes, how do you treat your water?	add chlorine or bleach
	Select all that apply.	solar disinfection
		other
13.	Is your source for drinking water ever used by animals?	yes
10.	lo your oouroe for armining water over accastly arminiate.	no
	1	1 ***
14.	In your dwelling is there a dedicated location for human solid	Vec
14.	waste/excreta? (e.g., toilet, latrine, designated area)	yes no
	Tractoroxorota: (o.g., tollot, latillo, dosignated area)	Ino
	Land of the state	
45	What is the highest level of education you have completed?	primary school
15.	Select one option.	secondary school
		Finished 10 th standard

				<u> </u>	college/university/professional	
					none	
					primary school	
	What is the highest level of education	n that your moth	er comple	ated?	secondary school	
16.	Select one option.	iii tilat your mour	Ci compi	ciou:	Finished 10 th standard	
10.	Colour one option.				college/university/professional	
					none	
					Hone	
				1		
			1.		ninerals, gas, oil, timber, coal	
			2.	crop production		
			3.	wildlife restau		
			4.		mal trade/market business	
			5.		r animal production business	
	Since this time last year what are the	e activities you	6.		ng, slaughterhouse, abattoir	
17.	have done to earn your livelihood?		7.		animal health care	
	Select all that apply.		8.	protected area		
			9.	hunter/trapper		
			10.		er/non-timber forest product collector	
			11.	migrant labore		
			12.		traditional healer, community health worker	
			13.	construction		
			14.	other:		
	nore than one activity was selected, no the activity number from the above			_	e most time since this time last year? *	
				r (non-governm		
				non-governmen	it)	
	Which best describes your job posi	tion?		r (Government)		
19.	Select one option.	uon:		(Government)		
	Coloct circ optioni				ndependently (Skip to question 28)	
			Professi	onal (health wo	rker, teacher)	
			other:			
Village Distric	nere do you work? (If different from w c/Town/City t ce/State					
comple	iewer: Probe for landmarks or nearestetion of interview. cal History Section	st known site if a	rea unkno	own. GPS coord	inates to be identified and entered after	
1. 0.1	and a firm that a first state of the first state of				and a second	
	section, I'm going to ask you about a al or treatment providers.	ny iliness or sick	mess that	is not known of	recognized in the community, including by	
Heald	aror rreatment providers.				clinic/health center	
					hospital	
	Where do you usually get tro-times	t for modical re-	hloma?			
21.	Where do you usually get treatmen	i ioi medicai proi	DIEITIS?		mobile clinic	
	Select all that apply.				community health worker	
					traditional healer	
<u></u>	1				dispensary or pharmacy	
		faces with hand		anness fatienna		
					or weakness (encephalitis)	
					d to injury (hemorrhagic fever)	
	In your lifetime, have you ever had				or difficulty breathing (SARI)	
an unusual illness with any of the fever with mu					tnroat (ILI)	
22.	following symptoms (READ ONLY	fever with diarrh	nea or vor	niting		
	SYMPTOMS)	fever with rash				
	Select all that apply.	persistent rash		on skin		
		no (Skip to ques			9	
1		mptoms - desci	describe			

	In an a contract of								
23.	Since this time last year, have yo	ou had any of these symptoms?	yes						
			no (Skip to question 29)						
		fever with headache and severe fatigue	or weakness (encephalitis)						
		fever with bleeding or bruising not related to injury (hemorrhagic fever)							
		fever with cough and shortness of breat	h or difficulty breathing (SARI)						
	If yes, which ones?	fever with muscle aches, cough, or sore throat (ILI)							
24.	Select all that apply.	fever with diarrhea or vomiting	tinoat (IEI)						
	Select all triat apply.								
		fever with rash							
		persistent rash or sores on skin							
		yes but, none of these symptoms - desc	cribe						
			contact with sick people						
			contact with wild animals						
			contact with other animals						
	In your opinion, when you were s	sick what caused this sickness?	bad food or water						
25.	Select all that apply.	non, what oddood the diomicoo.	bad spirits/witchcraft						
	Ocicci all triat apply.								
			wound or injury						
			I don't know						
			other:						
	1		1						
26.		ny of the people you lived with had any of	yes						
	these symptoms?		No (skip to question 29)						
			No (skip to question 29)						
	1								
		fever with headache and severe fatigue							
		fever with bleeding or bruising not relate	ed to injury (hemorrhagic fever)						
		fever with cough and shortness of breat							
	If yes, which ones?	fever with muscle aches, cough, or sore							
27.			tilloat (IEI)						
	Select all that apply.	fever with diarrhea or vomiting							
		fever with rash							
		persistent rash or sores on skin							
		yes but, none of these symptoms - desc	cribe						
28.	Since this time last year, did any	one you lived with die from this illness?	yes						
20.	Cirios tino timo last year, ala arry	one you have with the from this limes.	no						
			·						
Mov	ement Section								
In this	section, I'm going to ask you abou	ut any travel you have done since this time I	ast year						
111 (1113	Have you traveled since this tim		dot year.						
29.			yes						
29.	If answer is no, skip to the next	section.							
			no						
	here have you traveled since this t								
Interv	iewer: Probe for landmarks or nea	arest known site if area unknown. GPS coor	dinates to be identified and entered after						
compl	etion of interview.								
Collec	t up to 6 locations.								
ш									
	e are more than six locations chec								
Do no	t collect additional location informa	ation.							
			work						
1	Why have you traveled?		visit family						
31.	Select all that apply.		moved						
	OGIEGE AII MALAPPIY.		religious reasons						
			LIAMOINUE FASENNE						

	holiday/vacation
	go to hospital/seek medical care
	go to market
	other:

Animal Contact Section In this section, I'm going to ask you about the animals in your life. If answered "no" under the "in your lifetime" column, then no answer is required under the "Since this time last year" column. Since this In your lifetime ... time last year. yes yes 32. Has an animal lived as a pet in or near your dwelling? no no yes yes 33. Have you handled live animals? no no yes yes 34. Have you raised live animals? no no yes yes 35. Have you shared a water source with animals for washing? no no don't know don't know yes yes 36. Have you seen animal feces in or near food before you have eaten it? no no yes yes Have you eaten food after an animal has touched or damaged it? 37. no no For example, chew marks or scratches don't know don't know yes yes 38. Do any animals come inside the dwelling where you live? no no yes yes 39. Have you cooked or handled meat, organs or blood from a recently killed animal? no no yes yes 40. Have you eaten raw or undercooked meat or organs or blood? no no yes yes 41. Have you eaten an animal that you knew was not well /sick? no no don't know don't know Have you found a dead animal and collected it to eat or share? yes yes 42. Select all that apply. no no yes yes 43. Have you found a dead animal and collected it to sell it? no no yes yes 44. Have you been scratched or bitten by an animal? no nο let someone else take over wash wound with soap and water The last time you were scratched, bitten or cut yourself while butchering or slaughtering, rinse wound with water 45. what did you do? bandage wound Select all that apply. visit doctor nothing - kept working never butcher or slaughter yes, but I don't know what they Do you think there are any risks associated with slaughtering or butchering when you 46. have an open wound? Interviewer: Do not read responses. yes, it can make you sick

yes, it can poison you

											yes, it diseas don't l other		you with	а
47.	Have you s	laughtered a	n anim	al?								yes no	ye no	
48.	Have you h	unted or trap	ped aı	n animal?)							yes no	ye no	
		Circle all headings where "yes" was answere d in question s above.	pet (32)	handle d (33)	raise d (34)	feces in or near food (36)	in hous e (38)	cooked / handle d (39)	eaten raw/ under- cooke d (40)	eate n sick (41)	found dead (42/43)	scratche d/ bitten (44)	slaugh - tered (47)	hunted/ trapped (48)
		Elephant												
		rodents/ shrews												
		bats												
		non- human primates												
	Ask which animals	birds												
49.	/mammals for each "yes" category.	carnivore s												
		ungulates												
		pangolins												
		Poultry/ot her fowl												
		goats/ sheep												
		swine												
		cattle/ buffalo												
		dogs												

			,			,		,	•							
		cats														
		•										l.				
	coffee, tea, or cocoa plants															
											nut trees					
											plantation)				
									-	oil seed	crops					
											od planta	tion				
										dry gra	ins					
50.	Which crops	are at this s	site?							sugar	ole or fruit	orono				
50.	Select all tha	at apply.									legume	crops				
										fiber	leguille					
										forages						
										cover c						
										fallow f						
										rubber						
										fruits o	r nuts					
											than 1 y					
										1-2 y						
-4	I I am I am I	(1	/ ! .				0			2-5 y						
51.	How long n	ave the crop	os / pia	intations	been gi	rowing i	nere?			5-10						
										11–20 21–30) y					
										Greater than 30 y						
										Orcat	CI tilali 50	, y				
										Less than 1 y						
							1-2 y									
52.	How frequen	tly are crops	e / nlar	ntations h	arvecte	A2					2–5 y					
52.	I low frequen	itiy are crops	o / piai	ilalions n	iai vesic	ou:				5–10 y						
										11–2						
	<u> </u>									21–30 y						
	1									rodor	rodents/shrews					
										bats						
											numan prir	mates				
53.	What wild an	imals live in	crops	/ plantat	ions?					birds	iaman pin	natoo				
				, 1-1-1-1-1						carni	vores					
										ungul						
										pange	olins					
	1															
							under	ground mi	ining (by	shafts	or tunnels	5)				
							open s	surface m	ınıng • /bi =-b ==	*****	· ···ota="\					
54.	What type of	work or ind	ustry is	s conduc	ted her	e?	riyarai	ulic mininç ring, pann	y (riigh p	olloctic	water)					
54.	Select one o	ption.	-				oilwo	nng, pann II/gas field	ing, or c	onecun	y					
							loggin									
							other	<u> </u>								
							coal coltan									
								nd or othe	er gemst	one						
tin																
55.	What produc	t(s) are extr	acted?	,			gold/s	ııver								
	Select one o	ριιση.					lead									
							oil /ga timbei									
							electri									
									necify)							
	other (please specify)															

56.	Do you live on the work site?			yes			
00.	Do you live on the work one:			no			
				·			
			<10				
			10–100				
E 7	To the best of vour knowledge, how many poople work	101–1000					
57.	To the best of your knowledge, how many people work	at this site?					
		1001–10,000					
			>10,000				
			<1 mo				
			1 mo-1 y				
58.	How long have you worked at this site?	ow long have you worked at this site?					
			>1 y–5 y				
			>5 y				
59.	le there an aite feed production?			yes			
59.	Is there on-site food production?			no			
			1				
			the company	,			
60.	If yes, who pays for the cost of growing the food crops?	•		1			
			the workers				
				_			
64	la there meet evallable for consumption?			yes			
61.	Is there meat available for consumption?			no			
				1			
		farmed onsite					
				tat			
		farmed and purchased fr		ities			
62.	If yes, where does the meat come from?	purchased from wholesa	e market				
02.	Select all that apply.	locally caught/hunted					
	,	bought frozen					
		don't know					
		GOTT KHOW					
	T						
63.	Is it possible to consume bushmeat/wild animal meat o	n or near the site?		yes			
00.	To the production to controlling bushining wind arminar moat o	ir or riodi tiro oito :		no			
	Is there a designated area for rubbish, including animal	waste from slaughter/but	ther and animal	yes			
64.	excrement?	aataa a.aaga., zat		no			
	CAGIGITION:			110			
				Total -			
65.	If yes, do people use the designated location for rubbis	h?		yes			
				no			
00				yes			
66.	Do any animals raid food supplies or destroy crops?			no			
			rodonto/obrowo				
			rodents/shrews				
			bats				
			non-human primates				
			birds				
			carnivores				
			ungulates				
	If yes, what animals?		pangolins				
67.							
	Select all that apply.		poultry/other fowl				
			goats/sheep				
			camels				
			swine				
			cattle/buffalo				
		dogs					
			cats				
			barriers around fields	i			
			barriers on individual				
			fire				
00	What is done to stop animals from raiding or destroying	food supplies?	poison				
68.	Select all that apply.	,	traps				
	Solost all that apply.		shooting				
			loud sounds				
			domestic/guardian ar	nimals			
				IUIS			
			flooding				

								nasing anima	ls out				
							no	othing					
	1												
							_	dents/shrews					
							non-human primates						
		s have you hunted sir	nce this time last y	ear?			birds						
69.	Select all tha	at apply.					carnivores						
							_	ngulates					
							civits						
							pa	angolins					
							S	snare					
							t	oow					
							ŀ	nands					
							ç	gun					
70.	Since this t	ime last year, what	methods have	you used to h	unt/trap animals	s?	r	nachete					
70.	Select all th	nat apply.					k	nife					
							r	net					
							C	age					
							t	rap					
							C	other					
									liv	е			
			for	for use of		for o	مام	for sale of	trappi	ng of	aulling of		
	What is		for	animal	for sale for	for sa			nuisa		culling of		
	the		consumption	products at	consumption	alive		animal	anim	nals	nuisance		
	purpose of		at home	home	•	mark	et	products	for trans-		animals		
	your								loca	tion			
_,	trapping	rodents/shrews											
71.	or	bats											
	hunting?	non-human											
	Select all	primates											
	that apply.	birds											
		carnivores											
		ungulates											
		pangolins											
			•		•			•					
Since	this time last	t year, when you hu	unt or trap:										
72.											yes		
12.	Are you ex	posed to blood?									no		
70			·u 0								yes		
73.	Have you b	een scratched or b	oitten?								no		
74.	Cinna thin t	: lt b		.41 1 4 - 1	كوام مدنور ما ادانييراد	,					yes		
74.	Since this t	ime last year, have	you seen an o	utbreak of dea	ia wiia animais?				•		no		
							rc	dents/shrews	S				
							ba	ats					
							no	on-human pri	mates				
75.		h wild animals?						rds					
	Select all tr	nat apply. (add spe	cies list)				Ca	arnivores					
								ngulates					
								angolins					
	I.						I P	goo					
							touc	h it to see if it	is still	fresh			
								her in the fore					
						<u> </u>		ke or cook in		act			
	Mbot do vo	u da whan van fina	ممل امسنمم مما	d (not in a tra	. or obot by one					5 31			
What do you do when you find an animal dead (not in a trap or shot by another hunter)?								it to pre	Jaie				
76.									tion				
								rt it to authori	ities				
								it to sell it					
							noth						
							othe	r					
	ı					1							
	How do voi	u transport a dead	animal, if vou ta	ke it?				vrapped					
77.	Select all th		, ,	-				ped in leaves	s or oth	er nat	tural		
			material										

		wrapped in plastic		
		on yourself / carry by har	nd	
		in a bag		
		in a basket		
78.	Do you use special protective equipment (e.g., shoes, masks, gloves)?		yes	
70.	Do you use special protective equipment (e.g., shoes, masks, gloves)?		no	
		shoes/boots		
	If you we will take a marker of the control of the	mask		
79.	If yes, which protective equipment? Select all that apply.	clothes		
	Select all that apply.	gloves		
		gown/apron		
		•		
		handling anima	als	
	NATION AND AND AND AND AND AND AND AND AND AN	slaughter		
80.	When do you use protective equipment? Select all that apply.	butcher		
	Select all that apply.	always on at w	ork	
		other:		